This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 21!

See attached form for additional information.

Interagency Report Control No.:

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 10-F-0002
CUSTOMER NUMBER: 439

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

Walter Reed Army Institute Of Research
Div. Of Veterinary Medicine
Building 511 Robert Grant Ave.
Silver Spring, MD 20910

Telephone: (301) -319-7100

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Atached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (**Attach additional sheets if necessary or use APHIS Form 7023A**)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasc such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	18	0	0	18
5. Cats					
6. Guinea Pigs	0	69	182	186	437
7. Hamsters	0	0	103	0	103
8. Rabbits	0	95	19	0	114
9. Non-human Primates	0	536	104	36	676
10. Sheep	0	11	109	53	173
11. Pigs	0	25	245	9	279
12. Other Farm Animals					
13. Other Animals					

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and ap Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary inc brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

(b)(6), (b)(7)c

DATE SIGNED
4 Dec 02

APHIS FORM 7023 (Replaces VS FORM 18-23 (OCT 88), which is obsolete.)
(AUG 91)

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 10-F-0002

2. Number 186 of animals used in this study.

3. Species (common name) Guinea Pig of animals used in the study.

4. Explain the procedure producing pain and/or distress.

Shigella vaccine candidates were evaluated by placing Shigella in the conjuctivae of guinea pigs' eyes, and then the severity of inflammation was scored.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The study of immune response to and protective efficacy of vaccine candidates directed against Shigella requires an accurate evaluation of the immune response raised by the administration of these vaccines. The use of analgesics, particularly opiates or narcotics, result in immunosuppression, which would invalidate the results of experiments testing immune responses as well as increasing the severity of the possible eye infection. Use of analgesics that are anti-inflammatory (e.g. aspirin) would also invalidate the model since we are studying a model for inflammation of epithelial cells by bacterial invasion.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

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1. Registration Number: 10-F-0002

2. Number 36 of animals used in this study.

3. Species (common name) Rhesus Macaques of animals used in the study.

4. Explain the procedure producing pain and/or distress.

The aerosol doses of *Francisella tularensis LVS* strain SCHU-S4 will be administered 9 weeks (day 63) following the vaccine administration. The aerosol challenge will consist of $1x10^4$ cfu delivered as an aerosol delivered over between 5 to 15 minutes (dependent upon Plethsysmography to ensure correct aerosol dose) using a face mask exposure system. The particle stream will be limited to 2 micron size for maximal alveolar deposition. The aerosol stream will be sampled using side-scatter laser photometry, particle size distribution using time of flight analysis, and for viable counts using a constant flow impinger collecting into BHI broth. The organisms will be diluted from GLP-produced JVAP/DVC *F. tularensis* strain SCHU-S4 the organisms will then be diluted directly from the vial to the correct dosage. A Chocolate agar plate is subsequently streaked from the tube used to deliver each challenge, for isolation and to demonstrate that the inoculum was not contaminated. The final cfu/ml achieved is determined from the colony counts on Chocolate agar. The suspensions will be prepared in the BSL-3 laboratory immediately prior to administration. The inoculations will be administered under strict ABSL-3 conditions using a class III glove box. Each animal will be exposed separately in an upright seated position within the BSC and the bag-in/bag-out port used for entry and egress of the animals. Following administration the remaining organism suspension will be cultured for definitive analysis.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The aerosol exposure of virulent tularemia is critical to assessing the relative efficacy of the different routes of inoculation of the LVS vaccine to protect against aerosol exposure. Because the measurement of efficacy in tularemia is dependent upon a lethal model it is important that the disease process be interfered with as little as possible. Analgesics will be used post challenge as appropriate but because of the potential for analgesics to contribute to or exacerbate the symptoms of shock; it is unwise to use them in this protocol. Therefore any analgesic use will be carefully evaluated through consultation with the consulting veterinarian. Because fever is a major statistical parameter being used to evaluate the animal response to both the vaccine and challenge non-steroidal anti-inflammatory drugs (NSAIDs) will not be used in this protocol. The PI consulted with the consulting veterinarian in the planning of this protocol. On annual reports and at the conclusion of the study, the PI will inform the Chair, IACUC, of the number of monkeys that experienced pain or distress, or were prematurely euthanized during the study.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency_____ CFR _____

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1. Registration Number: 10-F-0002

2. Number 9 of animals used in this study.

3. Species (common name) Swine of animals used in the study.

4. Explain the procedure producing pain and/or distress.

Piglet model for both emetic and lethal response to Staph endotoxins (SE). Piglets are dosed orally with SE. A determination is made of the value of various potential drugs for prophylaxis against emesis (vomiting) and lethal shock. In addition, an evaluation of how late the drugs can be administered after SE-challenge and still retain desired efficacy of response is determined.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The lethal shock that is induced by the lethal SE-challenge with the LD50 test in the positive control animals will necessarily cause pain to these animals. Positive controls are required to validate results. Analgesics would impact the physiological parameters, exacerbating the lethal shock or emesis induced by the SE and compromising analysis of collected data. If the experimental drugs proved their utility, the animals should experience relief, but should they not experience relief then that indicates failure of the drug and is necessary for that reason. In all circumstances, the animals will be under constant veterinary care and will not be subject to any unnecessary pain.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

Column E Explanation

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1. Registration Number: 10-F-0002

2. Number 53 of animals used in this study.

3. Species (common name) Sheep of animals used in the study.

4. Explain the procedure producing pain and/or distress.

In Experiment 3, animals will be exposed to CO at 3 different concentrations. Since there should be no difference between NO and lung only (LO) type exposures, LO exposure will be required. Since the biochemical marker of interest for CO exposures is blood carboxyhemoglobin, additional animals for tissue analysis are not required. The animal numbers for the NO_2 + CO exposures in Experiment 4 are the same as for Experiment 1 since there is the need for NO and LO exposures and also additional animals for tissue analysis.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The only unalleviated pain, stress, or distress that may occur, will occur as a direct result of NO_2 exposure, particularly occurring on a delayed basis (approaching 24-h post exposure). However, study endpoints include neither death or near-death. Rather a subtler physio-chemical endpoint (hypoxemia) is sought. Some substernal stress and bronchoconstriction may be experienced in a sheep exposed to the highest gas exposure equivalent considered (*ie*, 3000 ppm x 5 min = 15,000 ppm-min, which is similar to 500 ppm x 30 min = 15,000 ppm-min – Januszkiewicz *et al* 1992; Januszkiewicz and Mayorga, 1994). This experience may be better classified as "stress and discomfort", rather than pain (a bronchospastic response is more stressful than painful). However, therapeutic corrective measures for these few animals (*ie*, bronchodilators, hyperoxia, analgesics that coincidentally alter cardiopulmonary function, *etc*) could directly or indirectly mask the desired endpoint. The PI has consulted with the attending veterinarian or his or her designee in the planning of both alleviated and unalleviated painful procedures.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____